Anticapsular Antibody Requirements for Protection against Experimental *Haemophilus influenzae* Type b Bacteremia after Splenectomy

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Although asplenic individuals are at higher risk for infection with encapsulated bacteria, it is not known whether they require a higher concentration of anticapsular antibody than normal individuals do for protection against invasive disease caused by *Haemophilus influenzae* type b. At 21 days of age, rats were passively immunized with human hyperimmune serum globulin against *H. influenzae* b polysaccharide (or saline) after recovery from splenectomy or a sham operation. Starting at 18 h after immunization, rats received three intranasal inoculations of 10^7 CFU of *H. influenzae* b over the next 24 h. Of sham-operated rats given 0.75 or 3.0 µg of anticapsular antibody, 91 or 96%, respectively, were protected from bacteremia, whereas only 59 and 67% of similarly treated asplenic rats were protected ($P < 0.004$, control versus asplenic rats). A 12-µg antibody dose resulted in the complete protection of both groups. The magnitude of bacteria was significantly higher in the asplenic group at each dose of antibody. Thus, asplenic hosts may require a higher concentration of anticapsular antibody than normal individuals do for protection against invasive *H. influenzae* b disease.

Asplenic individuals are at increased risk for bacteremic infections with encapsulated bacteria such as *Streptococcus pneumoniae* and *Haemophilus influenzae* type b (7). A sufficient concentration of anticapsular antibody to these pathogens correlates with protection from bacteremic infection. Several lines of evidence have resulted in an estimate of approximately 0.05 to 0.15 µg/ml as the anticapsular antibody concentration in serum which correlates with protection from invasive *H. influenzae* b disease (11, 15; P. Anderson, Letter, J. Infect. Dis. **149**:1034, 1984). A polysaccharide vaccine derived from capsular material of *H. influenzae* b, a polymer of ribose, ribitol, and phosphate, has recently been licensed for use in normal children 2 to 5 years of age. Although based upon limited data, this vaccine has also been recommended for use in asplenic individuals (3). However, the estimate of anticapsular antibody concentration which correlates with protection was derived from studies of individuals with intact spleens. Asplenic individuals may require a larger number of antibody molecules per particle to effect efficient clearance from circulation (10, 16). Thus, it is unclear whether asplenic hosts require a higher concentration of serum anticapsular antibody to afford protection from invasive *H. influenzae* b disease. I used an animal model of *H. influenzae* bacteremia to compare the anticapsular antibody requirements for protection of asplenic and normal rats.

MATERIALS AND METHODS

A streptomycin-resistant variant of *H. influenzae* b Eag was used in all experiments (20). The organism was grown to mid-logarithmic phase in supplemented brain heart infusion broth and prepared for inoculation as previously described (17).

Anticapsular antibody. The anti-*H. influenzae* b capsule antibody used for passive immunization was bacterial polysaccharide immune globulin (lot no. 22284, Massachusetts Department of Health) which was provided by George Siber.

This immunoglobulin G (IgG) preparation contains 367 µg of antibody to *H. influenzae* b capsular polysaccharide per ml (as well as a high titer of antibody to certain pneumococcal and meningococcal capsular antigens) (2). It has been used for successful passive protection against *H. influenzae* b invasive infection in infant rats (2) and normal infants (18). It is well absorbed after intraperitoneal or subcutaneous injection to infant rats (2; G. Siber, personal communication). Serum antibody to *H. influenzae* b capsular antigen was determined blindly on coded serum specimens at Praxis Biologics, Inc., Rochester, N.Y., by using a radioimmunoassay with intrinsically tritiated polysaccharide (4) and the Bureau of Biologics reference serum. The lower limit of detection was 0.10 µg/ml.

Animal model. Natural litters of outbred Sprague-Dawley rats were obtained from Charles River Breeding Laboratories, Wilmington, Mass. At 13 to 15 days of age, pups were given ether anesthesia and subjected to either splenectomy or a sham operation consisting of entry to the peritoneum and visualization of the spleen. At 20 days of age, rats were injected subcutaneously with 0.2 ml of phosphate-buffered saline containing 0, 0.75, 3, or 12 µg of anti-polyribosyl ribitol phosphate antibody (diluted bacterial polysaccharide immune globulin). At 18 h later, blood was obtained and pooled from five to seven asplenic or sham-operated rats at each dose of antibody. Rats were given three atraumatic 0.05-ml intranasal inoculations containing 10^7 CFU at 18, 24, and 42 h after the subcutaneous administration of antibody. Blood (0.025 ml) was obtained after tail vein puncture for culture on supplemented brain heart infusion agar containing streptomycin (500 µg/ml) at 48 and 72 h after the initial intranasal inoculation (17).

Statistics. Continuous data were compared by using Student's *t* test, and discontinuous data were compared by using Fisher's two-tailed exact test, with the aid of the Epistat program (T. Gustafson, Round Rock, Tex.).

RESULTS

As assessed at 18 h after the subcutaneous injection of immune globulin (at the time of intranasal inoculation with bacteria), increased doses of administered antibody resulted in increased concentrations of serum anticapsular antibody (Table 1). The concentrations of serum anticapsular antibody in pooled sera from sham-operated rats and from asplenic rats given the same dose of antibody were similar (Table 1). As assessed by culture of a throat swab obtained 48 to 72 h after inoculation, the colonization rates in sham-operated rats (92 of 120; 77%) and in asplenic rats (98 of 129; 76%) were similar. My sampling technique may have failed to detect quantitative differences in numbers of colonizing organisms. Immune globulin administration had no discernible effect on the rates of pharyngeal colonization (data not shown).

Of rats not given antibody, 94% developed bacteremia. A dose of 0.75 μg (which resulted in anticapsular antibody level in serum of 0.1 to 0.2 μg/ml) was effective in preventing bacteremia in sham-operated rats (9% bacteremia) but was much less effective in asplenic rats (41% bacteremia; $P = 0.004$). After the administration of a fourfold-higher dose of immune globulin, 24% of asplenic rats had detectable bacteremia, compared with 4% of normal rats ($P < 0.004$) (Table 1). Complete protection was observed only after a 12-μg dose of anticapsular antibody. The magnitude of bacteria was significantly higher in asplenic rats than in normal rats at each dose of anticapsular antibody (Table 2). Of 87 asplenic rats given phosphate-buffered saline or 0.75 μg of antibody, 3 were dead by 72 h, compared with no death among 79 control rats ($P = 0.14$; Fisher exact test).

DISCUSSION

I used an animal model which simulates natural *H. influenzae* b invasive disease in that bloodstream invasion and bacteremia follow intranasal inoculation. In this way, I sought to avoid the artificial situation of intravenous or intraperitoneal bacterial inoculation for which changes in bacterial inoculum size might alter the results and lead to

TABLE 1. Effect of passive immunization with anticapsular antibody on protection from *H. influenzae* b bacteremia of asplenic and control infant rats

Pretreatment	Mean concn (range) of serum anti-PRP Ab (μg/ml)[a]	No. of protected rats/no. of inoculated rats (%)[b]	
		Asplenic	Sham operated
Phosphate-buffered saline	<0.10	2/25 (8)	1/28 (4)
Antibody (μg)			
0.75	≤0.12 (<0.1–0.2)	19/32 (59)[c]	29/32 (91)[c]
3.0	0.33[d] (0.2–0.5)	37/55 (67)[e]	47/49 (96)[e]
12	2.83[f] (2.8–3.1)	24/24 (100)	16/16 (100)

[a] PRP, Polyribosyl ribitol phosphate; Ab, antibody. Serum was obtained at the time of initial intranasal inoculation with bacteria, 18 h after subcutaneous injection of immunoglobulin, and pooled from five to seven similarly treated rats. The range was derived from measurements of 4 to 10 independent samples of pooled sera at each pretreatment dosage.

[b] Protected rats had no bacteremia. Limit of detection, 40 CFU/ml of blood, measured 48 h after inoculation.

[c,e] $P = 0.004$ by Fisher's two-tailed exact test.

[d] Means (95% C.I.), 0.34 (0.13 to 0.55) for asplenic group and 0.31 (0.09 to 0.60) for sham group.

[f] Means (95% C.I.), 3.0 (2.6 to 3.5) for asplenic group and 2.8 (1.9 to 3.7) for sham group.

TABLE 2. Magnitude of *H. influenzae* b bacteremia after passive immunization of asplenic and control infant rats

Pretreatment	Concn[a] of bacteria in:		P[b]
	Asplenic rats	Sham operated rats	
Phosphate-buffered saline	5.56 ± 1.47	3.81 ± 1.11	<1 × 10⁻⁵
Antibody (μg)			
0.75	3.36 ± 2.24	1.72 ± .47	1.4 × 10⁻⁴
3.0	2.25 ± 1.44	1.65 ± 0.36	6.1 × 10⁻³

[a] Expressed as $\log_{10}$ of the geometric mean of CFU per milliliter of blood (± the standard deviation). The limit of detection was 40 CFU/ml (geometric mean = 1.60). The lower limit of detection was entered for animals without detectable bacteremia.

[b] By Student two-tailed t test, comparing asplenic and sham-operated groups.

difficulties in interpreting the data. The size of the intranasal inoculum was probably not critical, as Moxon et al. (12) observed a similar mortality over a fourfold logarithmic range (10^4 to 10^8 CFU) of intranasal inoculum of *H. influenzae* b given to asplenic rats. By subcutaneous injection of antibody to achieve passive immunization, I entirely avoided the peritoneum which may have been altered by the previous surgical procedure. The endpoint, detection of bacteremia, is a clinically relevant parameter measuring the net effect of in vivo bacterial multiplication and the efficacy of host clearance mechanisms. I did not examine cerebrospinal fluid to detect meningitis. As previous studies have shown that the development of meningitis correlates directly with the magnitude and duration of *H. influenzae* b bacteremia (13), one would predict that many of the asplenic bacteremic rats, as well as sham-operated rats not pretreated with antibody, will develop meningitis.

The anticapsular antibody source was purified human immune serum globulin which contained >95% IgG and only traces of IgM and IgA (2). This material promotes complement-dependent bacteriolysis, as well as phagocytosis by polymorphonuclear leukocytes (19). Although I gave human IgG to rats, this study, as well as a previous study using 5- to 6-day-old rats (2), has shown that the antibody requirement for protection of animals with intact spleens, i.e., ≤0.1 to 0.2 μg/ml, is similar to that for protection of humans from infection (0.06 to 0.10 μg/ml) (15).

To my knowledge, this is the first demonstration of an increased antibody requirement for protection of an asplenic host against bacteremia. The data suggest that a greater than fourfold-higher concentration of anticapsular antibody in serum is required to afford protection from *H. influenzae* b bacteremia in asplenic subjects. However, protection from bacteremic infection is a complex and dynamic issue balancing bacterial multiplication against specific and nonspecific bacterial clearance mechanisms which are present or may be stimulated by antigens of invading bacteria. Opsonophagocytosis appears to be a more important mechanism for bloodstream clearance of *H. influenzae* b than complement-mediated bacteriolysis (13a, 21). Because the liver, the major site of pneumococcal clearance in asplenic animals (6, 10), is a less efficient reticuloendothelial organ than the spleen, a requirement for an increased level of opsonization for efficient hepatic clearance (10, 16) may explain the observed increase in antibody requirements. My data are consistent with those of Hosea et al. (10) and Brown et al. (6) who found that the defect in the clearance of intravenously injected pneumococci in asplenic guinea pigs is correctable by immunization. If asplenic hosts also require a higher

antibody concentration in serum than normal individuals do for protection against pneumococci, this finding might explain, in part, the vaccine failure reported in individuals with functional or anatomic asplenia (1, 5). Additionally, asplenic individuals may have impaired antibody responses to pneumococcal polysaccharide vaccine (9).

There are no data available on the anticapsular antibody concentration which correlates with the protection of asplenic humans (including individuals with functional asplenic states such as sickle-cell anemia) from *H. influenzae* b bacteremia. Antibody to other bacterial surface antigens, such as lipooligosaccharide or outer membrane proteins, may contribute to protection from bacteremic infection. Nonetheless, it is likely that asplenic humans require a higher anticapsular antibody concentration than normal individuals do for protection against *H. influenzae* b bacteremia. The occasional occurrence of *H. influenzae* b invasive disease in sickle-cell disease patients older than 5 years (14, 22) and an *H. influenzae* b vaccine failure (8) tend to support this hypothesis. This problem may be compounded by an apparent hyporesponsiveness of young children with sickle-cell disease to *H. influenzae* b polysaccharide vaccine (L. Rubin, D. Voulalas, L. Carmody, and G. Karayalcin, Pediatr. Res. 1987, 21:338A). Thus, one cannot assume that an *H. influenzae* b vaccine which shows efficacy in a population of normal individuals will also be efficacious in asplenic individuals.

ACKNOWLEDGMENTS

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